MAYO CLINIC

The Many Faces of Cobalamin (Vitamin B_{12}) Deficiency

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Abstract

Although cobalamin (vitamin B_{12}) deficiency was described over a century ago, it is still difficult to establish the correct diagnosis and prescribe the right treatment. Symptoms related to vitamin B_{12} deficiency may be diverse and vary from neurologic to psychiatric. A number of individuals with vitamin B_{12} deficiency may present with the classic megaloblastic anemia.

In clinical practice, many cases of vitamin B_{12} deficiency are overlooked or sometimes even misdiagnosed. In this review, we describe the heterogeneous disease spectrum of patients with vitamin B_{12} deficiency in whom the diagnosis was either based on low serum B_{12} levels, elevated biomarkers like methylmalonic acid and/or homocysteine, or the improvement of clinical symptoms after the institution of parenteral vitamin B_{12} therapy. We discuss the possible clinical signs and symptoms of patients with B_{12} deficiency and the various pitfalls of diagnosis and treatment.

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Several scientific articles and textbooks have described the clinical presentation of patients with cobalamin (vitamin B_{12}) deficiency.[1,2] After the classic presentation of Addison-Biermer disease with megaloblastic anemia, many generations of doctors have been educated with the view that vitamin B_{12} deficiency exclusively presents itself with this type of anemia. Additional cases have been reported in which neurologic abnormalities were the main presenting symptom, with subacute combined degeneration of the spinal cord as one of the most feared manifestations,[3] often leading to permanent disability. Lindenbaum et al[4] reported a large series of 40 patients who had neurologic symptoms or psychiatric disorders caused by vitamin B_{12} deficiency but who had no anemia or macrocytosis. Psychiatric symptoms may vary from depression to mania, psychosis, and occasionally suicidal thoughts (Supplemental Table 1, available online at http://mcpiqojournal.org).[5] The reason why some patients mainly present with megaloblastic anemia and others with neurologic symptoms remains unknown.

Laboratory investigations with the establishment of low serum B_{12} levels and elevated levels of methylmalonic acid (MMA) are the cornerstone of diagnostics, but normal levels of serum B_{12} and MMA do not exclude symptomatic B_{12} deficiency. In clinical practice, many cases of B_{12} deficiency are overlooked or sometimes even misdiagnosed because of misconceptions and misbeliefs among health care professionals. We have summarized the most frequently encountered misconceptions and misbeliefs regarding vitamin B_{12} deficiency in Table 1.

In this review, we discuss a number of typical patients who were seen at our outpatient clinic, and we summarize the possible clinical signs and symptoms of patients with vitamin B_{12} deficiency and the pitfalls of diagnosis and treatment.

CASE DESCRIPTIONS

Patient A

Patient A is a 55-year-old woman admitted to the psychiatry department of our hospital because of depression. In addition to symptoms related to her depression, she reported pain in the lower legs, paresthesia and numbness in the feet, and difficulty walking. Her

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TABLE 1. Frequently Reported *Misconceptions and Misbeliefs* Regarding Vitamin B_{12} Deficiency

Diagnosis: You do not have vitamin B_{12} deficiency because
• You have no anemia
• You have no macrocytic anemia
• Your serum vitamin B12 level is within the reference range of 140 to 450 pmol/L
• Your serum vitamin B12 level is only moderately low
• Your serum vitamin B12 level may be low, but your plasma level of methylmalonic acid is completely normal
• Vitamin B12 deficiency only occurs in elderly people
• Vitamin B12 deficiency never occurs in children
Treatment
• Oral therapy is as good as or even better than painful injections to alleviate neurologic symptoms
• You may stop treatment because your serum vitamin B_{12} level has normalized
• You must stop treatment because your serum vitamin B_{12} level is elevated
• You must stop after 5 injections because vitamin B_{12} can have serious adverse effects
• You must stop the injections because your symptoms have worsened
• We must measure your serum vitamin B_{12} level after 3 injections to see whether the treatment is successful
• We must stop vitamin B_{12} injections now that you are pregnant

history included Graves disease, for which she had undergone thyroidectomy several years earlier and was subsequently prescribed levothyroxine. We were invited for consultation because of slightly elevated free thyroxine levels. The patient had no symptoms suggestive of thyrotoxicosis. On examination, she had signs of severe peripheral neuropathy of the lower legs (absent reflexes, superficial and deep sensation) and several skin lesions compatible with vitiligo. Laboratory studies (also summarized in Supplemental Table 2, available online at http://mcpiqojournal.org) (reference ranges provided parenthetically) revealed a hemoglobin level of 7.0 mmol/L (>7.5 mmol/L) and mean corpuscular volume of 105 fl (85-98 fl). Her serum vitamin B_{12} level was 51 pmol/L (145-450 pmol/L). These findings prompted the diagnosis of pernicious anemia caused by vitamin B_{12} deficiency, and hydroxocobalamin injections were initiated immediately. Within 3 to 4 weeks, the patient reported that her symptoms gradually lessened, and her walking capacity improved. Additional laboratory tests revealed the presence of antibodies against parietal cells and intrinsic factor (IF). Six weeks later, the attending psychiatric resident ordered an additional serum vitamin B_{12} measurement. Because the serum vitamin B_{12} level was greater than 1476 pmol/L, hydroxocobalamin injections were stopped after consultation with an internal medicine resident. The patient was discharged home 3 months later, but no follow-up appointment at our outpatient clinic was made. The discharge letter did not mention the diagnosis of pernicious anemia, nor the treatment with hydroxocobalamin injections. This exclusion went unnoticed until 8 months later, when we realized she was lost to follow-up and invited her for a follow-up visit. She presented at our outpatient clinic shortly thereafter and reported a severe increase of her symptoms. One month earlier, her primary care physician had ordered measurement of her serum vitamin B_{12} level, which was 251 pmol/L; he did not restart treatment because he was unaware of the earlier findings. We strongly advised the patient to recommence hydroxocobalamin injections, which she continued twice weekly for the following 2 years. Gradually, her symptoms abated. Two years later, there were still bouts of pain, numbness, and paresthesia, but she was able to walk supported only by a walking stick. This case is a clear example of pernicious anemia in the constellation of a polyglandular autoimmune syndrome. Unwarranted cessation of therapy may lead to severe worsening of neurologic abnormalities and irreversible damage.

Patient B

Patient B is a 17-year-old girl who had a 2-year history of fatigue, sleepiness, numbness in her hands, dizziness, exertional dyspnea, and

problems with concentrating and finding the correct words while in conversations. Table 2 summarizes her most important symptoms. Because her mother had presented with symptomatic vitamin B_{12} deficiency 6 months earlier, she was tested as well. Clinical examination did not reveal major abnormalities. Laboratory examination yielded a total serum vitamin B_{12} level of 112 pmol/L, with an intermediate level of holotranscobalamin (holoTC) of 54 pmol/L (40-125 pmol/L) and a low total haptocorrin concentration of 162 pmol/L (203-596 pmol/L). Her MMA value was 178 nmol/L (<300 pmol/L), homocysteine level was 11 pmol/L (<10 pmol/L), and folate concentration was 19 nmol/L (10-36 nmol/L). Antibodies against parietal cells, IF, and transglutaminase/gliadin were absent. Because of our clinical suspicion of vitamin B_{12} deficiency, she was treated with hydroxocobalamin, 1000-μg injections twice weekly, with a beneficial effect on her symptoms (Table 2). As can be appraised from her symptom list, many improved considerably or resolved completely. This case illustrates the clear clinical syndrome of vitamin B_{12} deficiency despite intermediate holoTC and normal MMA levels.

Patient C

Patient C is a 15-year-old girl. She had no specific symptoms. Her exercise capacity was normal, and her school grades were fine. Because her brother was diagnosed as having symptomatic vitamin B_{12} deficiency at age 17 years and was subsequently successfully treated, she was referred for evaluation. Physical examination yielded no abnormalities. Laboratory investigation revealed a serum vitamin B_{12} level of 94 pmol/L initially and 114 pmol/L on repeated testing, MMA value of 218 nmol/L, homocysteine concentration of 78.4 μmol/L, and folate level of 6.7 nmol/L. Extensive evaluation showed no additional abnormalities, especially no signs of celiac disease, or antibodies against parietal cells or IF. Whole-exome sequencing of genes related to metabolic disorders yielded only the well-known homozygous c.677C>T mutation in the gene coding for methylenetetrahydrofolate reductase. It is known that people with this mutation have increased risk of mild hyperhomocysteinemia with vitamin B_{12} and/or folate deficiency.[6] This patient had no clinical symptoms related to vitamin B_{12} deficiency and a severely elevated homocysteine level, but the genetic mutation is not considered to be the sole

TABLE 2. Self-Reported Symptoms of Patient B Before and 1 and 6 Months After Initiation of Hydroxocobalamin Therapy[a]

Variable	Before treatment	After treatment	
		1 mo	6 mo
Numbness in hands	5	4	1
Difficulties focusing	5	4	3
Not being able to find the right words	6	4	0
Mood changes	7	6	0
Pain in mouth and tongue	6	6	4
Fatigue, weakness	9	9	0
Nausea, reduced appetite	8	6	0
Menstrual pains	9	9	8
Pain in joints	6	6	3
Dyspnea on exertion	6	8	1
Dizzy spells	7	5	0
Pale skin	5	5	4
Feeling cold	8	8	0
Muscle cramps	6	6	4
Stomach complaints, acidity	6	4	0

[a]Symptoms rated on a scale of 0 to 10, in which 0 = no symptoms and 10 = worst symptoms.

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cause of this patient's vitamin B_{12} deficiency and marked elevation of homocysteine.

Patient D

Patient D is a 33-year-old woman with a history of fatigue, inertia, indolence, paresthesia in her hands and feet, difficulties concentrating, problems with remembering things, and word finding disturbances. An acquaintance mentioned to her the possibility of vitamin B_{12} deficiency. Her primary care physician ordered a serum vitamin B_{12} measurement, which was 190 pmol/L, and subsequently advised her to start hydroxocobalamin injections. Her MMA and homocysteine levels were unfortunately not measured. After 5 weeks of treatment, her symptoms had decreased considerably. Her physician then ordered repeated serum vitamin B_{12} measurement, which revealed a value of more than 1476 pmol/L. Subsequently, the physician became concerned about possible vitamin B_{12} intoxication and asked the patient to stop treatment. Five to 6 weeks later, her symptoms had increased substantially. She was referred to our clinic for evaluation. Her main question was why this successful treatment was stopped. Hydroxocobalamin injections were restarted, in a frequency of twice weekly, which resulted in a gradual improvement of her symptoms. This case illustrates the difficulties of an incomplete diagnostic work-up and also the dangers of measuring serum vitamin B_{12} levels during parenteral administration. Hydroxocobalamin is not toxic, and successful treatment should not be stopped. Usually, the period between injections can be prolonged when all symptoms have disappeared.

Patient E

Patient E is a 68-year-old woman who was treated by a hematologist in another hospital for a period of 3 years because of macrocytic anemia (hemoglobin, 7.5 pmol/L; mean corpuscular volume, 110 fl). There was no history of alcohol abuse. Extensive evaluation revealed no other abnormalities, and her serum vitamin B_{12} value was 301 pmol/L. She had already been treated for several years with levothyroxine because of primary hypothyroidism. Myelodysplastic syndrome was diagnosed by bone marrow aspiration, and she received blood transfusions twice. In 2017, she experienced progressive pains in her legs and almost completely lost the feeling in her feet; she was not able to walk outside her house anymore. Even walking in her home was extremely difficult and painful. Additional laboratory examinations did not reveal any new abnormalities. She was admitted to the neurology department in another hospital and found to have severe polyneuropathy. Magnetic resonance imaging of the spinal cord showed symmetric bilateral high signal within the dorsal columns, suggestive of subacute combined degeneration of the cord; magnetic resonance imaging of the brain revealed minimal white matter lesions. Again, her serum vitamin B_{12} level was normal, as were thiamine, pyridoxine, and folate values. No signs or laboratory abnormalities consistent with connective tissue disease or other causes of polyneuropathy were found. Biochemical and microbiological evaluation of cerebrospinal fluid revealed no abnormalities. After 2 weeks of observation, her doctors decided to initiate hydroxocobalamin treatment. Two days later, results of additional testing were returned: her MMA level was 37,000 nmol/L, and her homocysteine value was 165 μmol/L. These findings confirmed the existence of severe vitamin B_{12} deficiency. Additional testing yielded a high titer of antibodies against IF and parietal cells. Because of uncertainty about the diagnosis, she was referred to our outpatient clinic 2 months later. No additional clues arose from history or physical examination. Repeated examination of the bone marrow specimen did not confirm a diagnosis of myelodysplastic syndrome and showed only a slight increase of erythropoiesis. The most likely diagnosis was Addison-Biermer disease and interference in the vitamin B_{12} assay by the IF autoantibodies. No serum samples were left to test this hypothesis. Six months after diagnosis and initiation of high-dose hydroxocobalamin treatment, the patient still had considerable neurologic damage, loss of sensations in her feet and legs, and inability to walk without the use of a rollator walker. The anemia has resolved completely. This case is a classic example of how the assay for serum vitamin B_{12} can be wrong because of interference of the assay by IF autoantibodies.

Patient F

Patient F is a 62-year-old woman with a body mass index of 36.6 kg/m^2. She underwent a

Roux-en-Y gastric bypass procedure 8 months earlier, which resulted in a 40-kg weight loss. During a routine visit to the outpatient clinic, she reported fatigue and difficulties in concentrating and executing difficult tasks. She took multivitamins that were prescribed by the physician in the bariatric surgery center. Before the operation, she underwent a laboratory evaluation that revealed the following: serum vitamin B_{12}, 303 pmol/L; folate, 15.4 nmol/L; and vitamin D, 59 nmol/L. At reassessment, her serum vitamin B_{12} concentration was 249 pmol/L, and her MMA level was 1380 nmol/L. Because of the suspicion of vitamin B_{12} deficiency, treatment with hydroxocobalamin injections, 1000 μg twice weekly, was initiated, after which most of her symptoms disappeared within 6 to 8 weeks. This case illustrates that vitamin B_{12} levels within the reference range do not exclude symptomatic vitamin B_{12} deficiency.

Discussion

We have described the disease spectrum of a number of patients who attended our outpatient clinic with possible vitamin B_{12} deficiency in whom the diagnosis was either based on low serum vitamin B_{12} levels, elevated biomarkers like MMA and/or homocysteine, or the improvement of clinical symptoms after the institution of parenteral vitamin B_{12} therapy. Altogether, these cases show the considerable heterogeneity of vitamin B_{12} deficiency. Not all cases were easy to recognize or diagnose. The most prevalent symptoms of vitamin B_{12} deficiency are neurologic, such as paresthesia in hands and feet, muscle cramps, dizziness, cognitive disturbances, ataxia, and erectile dysfunction, as well as fatigue, psychiatric symptoms like depression, and macrocytic anemia.[1,2,7–10] However, there is evidence that in the Western world as well as China, less than 20% of people with demonstrable low serum vitamin B_{12} levels have macrocytic anemia.[11–14] Although the demonstration of low serum vitamin B_{12} levels is considered diagnostic, there is a poor correlation between these levels and symptoms,[8,9,15] and even people with vitamin B_{12} levels below 140 pmol/L may not have symptoms.[16] This factor sheds a different light on the discussion regarding appropriate cutoff levels for serum vitamin B_{12} and related parameters. Some investigators suggest that it is necessary to establish different reference cutoffs according to age and the applied analytic method.[17] However, serum vitamin B_{12} tests also may fail because many people with symptoms related to cobalamin deficiency may have serum vitamin B_{12} levels above the lower reference level of 140 pmol/L.[18,19] Although several factors may be of influence, in a considerable number of cases this issue can be caused by the earlier use of oral supplementation with multivitamins or high-dose oral vitamin B_{12} preparations.[20] It has been reported that even a dose of 10 μg/d can increase vitamin B_{12} levels to more than 200 pmol/L in elderly individuals (>65 years).[21] Oral supplementation may increase the serum vitamin B_{12} level but often not enough to replenish the vitamin B_{12} levels in the tissues[21] unless very high doses (1000-2000 μg/d) are used.

BIOCHEMISTRY AND DIAGNOSIS

Vitamin B_{12} deficiency may be easily overlooked in patients when only total serum vitamin B_{12} is used as a status marker.[18,22–25] Many investigators therefore advocate measuring one or both of the additional functional biomarkers MMA and homocysteine to establish vitamin B_{12} deficiency. Especially in people with so-called borderline vitamin B_{12} levels, ie, those between 140 and 300 pmol/L, elevated MMA and/or homocysteine values may aid in establishing a possible diagnosis of deficiency.[18,26,27] The mechanism by which these biomarkers may demonstrate cobalamin deficiency can be derived from the role of vitamin B_{12} in our body. Vitamin B_{12} is a pivotal cofactor in 2 enzymatic reactions. Its deficiency will impair the proper function of these enzymes and lead to accumulation of the substrate. Methylmalonyl—coenzyme A (CoA) mutase, which catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA, is one of the vitamin B_{12}—dependent enzymes, and impaired function will cause elevations of methylmalonyl-CoA and thereby MMA after cleavage of CoA. The other vitamin B_{12}—dependent enzyme is methionine synthase, which regenerates methionine from homocysteine, and impaired activity as the consequence of vitamin B_{12} deficiency will lead to accumulation of homocysteine.[28,29] From this process, it can be understood how low tissue levels of cobalamin

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will increase MMA and homocysteine, and a clear relationship between serum vitamin B_{12}, MMA, and homocysteine has been described in the National Health and Nutrition Examination Survey population.[29] Nevertheless, the sensitivity and specificity of elevated MMA and/or homocysteine levels in patients with symptoms associated with vitamin B_{12} deficiency are unknown. Also, it has been documented that MMA levels are elevated in people with severely impaired renal function.[30] Similarly, elevated homocysteine values can also be the consequence of folate or vitamin B_6 deficiency, as well as impaired renal function, hypothyroidism, and certain medications.[6] In a separate study, we calculated from the National Health and Nutrition Examination Survey and Lifelines epidemiological studies that MMA and/or homocysteine levels are elevated above current reference values (>300 nmol/L and 10 μmol/L, respectively) in only 73% of people with low serum vitamin B_{12} levels of less than 140 pmol/L and in 28% of patients with serum vitamin B_{12} levels between 140 and 300 pmol/L (B.H.R.W., H.J.C.M.W., J.E. Kootstra-Ros, et al, unpublished data, 2019). Other studies have confirmed that normal levels of MMA may be measured even in situations of very low vitamin B_{12} levels.[31] In addition, there are isolated reports that serum vitamin B_{12}, homocysteine, and MMA levels are unreliable predictors of vitamin B_{12}–responsive neurologic disorders.[32] Genetic studies have found that in addition to mutations in the *MMUT* gene (for expansion of gene symbols, use search tool at www.genenames.org) associated with methylmalonylaciduria, single-nucleotide polymorphisms in *HIBCH* and *ACSF3* have been associated with MMA levels.[33,34] Similarly, serum vitamin B_{12} levels may also be influenced by specific polymorphisms or mutations.[35–37] One of these factors is the gene *FUT2*, in which the *FUT2* secretor variant genotype AA is associated with a 10% to 25% higher total and haptocorrine-bound vitamin B_{12} level but not holoTC/active vitamin B_{12}.[38]

During recent years, the clinical usefulness of measuring holoTC as a screening for vitamin B_{12} status has received attention. HoloTc is the biologically active form of vitamin B_{12} in plasma. Some studies have suggested that holoTC has a better diagnostic accuracy than total serum vitamin B_{12} level.[39,40] However, holoTc also has a large window with indeterminate levels,[27] and the reference values strongly depend on the assay method used.[39] It has been suggested that patients with holoTC levels between 23 and 75 pmol/L require further testing of MMA levels to document, or rule out, true vitamin B_{12} deficiency.[41] Also, approximately 63% of people with low holoTc levels (<27 pmol/L, indicative of true deficiency) have normal levels of MMA, while 9% of patients with holoTC values above 63 pmol/L have elevated MMA levels (defined as >300 nmol/L). This issue raises questions about whether holoTC measurement is really superior to measurement of total serum vitamin B_{12} plus MMA for determining vitamin B_{12} deficiency[41] but also indicates that MMA is a poor indicator of vitamin B_{12} deficiency. Indeed, in another study, both serum vitamin B_{12} and holoTC levels were weak predictors of abnormal MMA levels.[23]

A more mathematical approach toward establishing vitamin B_{12} deficiency was proposed by Fedosov et al,[42,43] who calculated a single combined indicator of vitamin B_{12} status in which levels of total serum vitamin B_{12}, holoTC, MMA, and homocysteine are taken into account. This is an elegant approach to making a proper diagnosis, but its validation in the context of both functional vitamin B_{12} deficiency and systematically evaluated response to vitamin B_{12} supplementation therapy (see subsequent discussion) is required.

ASSAY INTERFERENCE

Several published case reports have shown functional vitamin B_{12} deficiency in patients with apparently normal serum vitamin B_{12} levels. In some of these patients, interference of serum vitamin B_{12} assays by IF antibodies has been demonstrated,[44–47] and it has been reported that assays fail to measure low total vitamin B_{12} concentrations in some samples because of an unknown artifact.[48] Carmel and Agrawal[48] reported that in 25% of patients with pernicious anemia, the assay may have produced false-normal values. We strongly believe that our patient E fits the description of interfering anti-IF antibodies. She had overt macrocytic anemia, initially diagnosed as myelodysplastic syndrome. Repeated serum vitamin B_{12} measurements

yielded normal results, and in the following 3 years, she had development of symptoms compatible with severe polyneuropathy and nerve damage, signs of subacute combined degeneration of the spinal cord. After both MMA and homocysteine levels were found to be grossly elevated, it was realized that she had severe vitamin B_{12} deficiency. An additional factor that may have added to her protracted course was that she had been treated with high doses of folate. It is well known that folate therapy may mask anemia, and not treating with cobalamin may accelerate neurologic damage in people with vitamin B_{12} deficiency.[49]

POSSIBLE CAUSES OF VITAMIN B_{12} DEFICIENCY

The polyglandular autoimmune syndrome is easily recognizable as a cause of deficiency in patients A and E. Polyglandular autoimmune syndromes are characterized by a number of (endocrine) diseases in which autoantibodies directed against certain organs or cell constituents can be demonstrated in the blood.[50–53] Table 3 summarizes the most prevalent disorders, varying from autoimmune thyroid disease (Hashimoto or Graves disease) to type 1 diabetes, Addison disease, and vitiligo. In the situation of vitamin B_{12} deficiency, both antibodies against IF and antibodies against parietal cells can be found, although it must be realized that the sensitivity of these measurements is low. One study found that only 55% of people of Western European descent with documented pernicious anemia had anti-IF antibodies.[54] However, this was not a very recent study, and methodology to demonstrate antibodies may have changed. Studies that assessed different assays to measure anti-IF antibodies have yielded discrepant results.[54–56] Conversely, some investigators have argued that testing for gastric parietal cell antibodies is an appropriate screening test for pernicious anemia.[57]

Other causes that can lead to vitamin B_{12} deficiency are summarized in Supplemental Table 3 (available online at http://

TABLE 3. Spectrum of Polyglandular Autoimmune Syndrome

Organ	Disease	Antigen
Thyroid	Hashimoto disease	Thyroid peroxidase
	Graves disease	Thyrotropin receptor, thyroid-stimulating immunoglobulins
Pancreas	Type 1 diabetes	Glutamic acid decarboxylase 65, Islet antigen 2, zinc transporter 8
Adrenal glands	Addison disease	21-Hydroxylase
Gonads	Autoimmune oophoritis	17α-Hydroxylase, side-chain cleavage enzyme
	Premature menopause	Aldehyde (retinal) dehydrogenases, selenium-binding protein 1
	Autoimmune orchitis	Various antigens of sperm cell or testicular basement membrane
Pituitary gland	Lymphocytic hypophysitis	Various antigens suggested
Parathyroid glands	Hypoparathyroidism[a]	NACHT leucine-rich repeat protein 5
Intestine	Celiac disease	Gluten
Stomach	Atrophic gastritis	Parietal cells, intrinsic factor
Liver	Autoimmune hepatitis	Cytochrome P450 1A2, 2A6
	Sclerosing cholangitis	Unknown
	Primary biliary cirrhosis	Mitochondrial antigens, like subunits (E2) of the pyruvate dehydrogenase complex
Blood	Thrombocytopenia	Platelet surface glycoproteins
	Hemolytic anemia	Various antigens
Skin	Vitiligo	Tyrosinase
Hair follicles	Baldness/alopecia	Tyrosinase

[a]Often in combination with mucocutaneous candidiasis.

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mcpiqojournal.org). One important cause is celiac disease, in which gluten hypersensitivity will cause atrophy of the intestinal villi and consequently malabsorption, not only for vitamin B_{12} but also for a variety of other vitamins. Celiac disease is one of the disorders in which vitamin B_{12} absorption may be restored after dietary adjustment with complete avoidance of gluten. Other factors that may contribute to vitamin B_{12} malabsorption, hence deficiency, are certain medications like metformin and proton pump inhibitors,[58,59] H_2 receptor blockers, and antacids. Metformin use was associated with an increase in MMA and worsening neuropathy score in patients with type 2 diabetes.[60] In addition, vitamin B_{12} malabsorption may be more prominent in older individuals, rendering these people at increased risk of symptomatic vitamin B_{12} deficiency.[61,62]

Inadequate dietary intake is another important cause of vitamin B_{12} deficiency. Because vitamin B_{12} is mainly present in food products from animal origin, vegetarians and vegans are at especially high risk for development of vitamin B_{12} deficiency.[63] Finally, a new group of vitamin B_{12}–deficient individuals is rapidly developing as the number of people undergoing bariatric surgery worldwide is increasing.[64,65] Bariatric surgery has become one of the major treatments for (morbid) obesity, and reduced production of both IF and gastric acid, as well as reduced intake of vitamin B_{12}–containing foods, contribute to the development of vitamin B_{12} deficiency (patient F). A recent study from The Netherlands reported a 60% incidence of post–bariatric surgery vitamin B_{12} deficiency, defined as a serum MMA level greater than 300 pmol/L.[66] Interestingly, these investigators also reported that positive results of parenteral vitamin B_{12} administration were reported by patients without functional vitamin B_{12} deficiency (MMA <300 nmol/L), suggesting that supplementation itself, regardless of the actual vitamin B_{12} status, improves clinical symptoms.[66] Finally, severe cases of vitamin B_{12} deficiency have been reported in young individuals using nitrous oxide as a recreational drug.[67–69]

FAMILIAL/GENETIC CAUSES OF VITAMIN B_{12} DEFICIENCY

The literature on familial cases of vitamin B_{12} deficiency is limited. Classically, the Imerslund-Gräsbeck syndrome is mentioned as an example of a genetic cause of vitamin B_{12} deficiency due to selective malabsorption, but its prevalence is very low. Other causes may be mutations in the gene for intrinsic factor,[70] genes encoding the vitamin B_{12} transporting transcobalamins,[71,72] and genes involving intracellular vitamin B_{12} metabolism. The list of single-nucleotide polymorphisms associated with serum vitamin B_{12} concentration is gradually increasing, and a recent review reported 59 gene polymorphisms found in a wide variety of populations, although the majority were reported in people of Western European descent.[37] Recent studies have found an association between homozygosity of the TT methylenetetrahydrofolate reductase C677T genotype and vitamin B_{12} deficiency.[73–75] In the near future, dedicated evaluation of genetic mutations in genes that are associated with vitamin B_{12} metabolism, for instance with whole-exome sequencing, may shed light on familial cases.

CONSEQUENCES OF MATERNAL AND INFANT LOW B_{12} STATUS

Maternal vitamin B_{12} deficiency during pregnancy may be associated with an increased incidence of neural tube defects and brain development retardation,[76] as well as preterm birth and low birth weight.[77] Wnt signaling has been reported to be disrupted in the developing cerebellum of the offspring of vitamin B_{12}– and folate-deficient female rats[78] and is associated with long-term disabilities of behavior and memory.[79] Ethnic differences in vitamin B_{12} levels have been observed in pregnancy, possibly related to differences in intake of animal-derived foods.[80] Children born to mothers with normal to high folate levels and low serum vitamin B_{12} values have higher truncal adiposity and insulin resistance,[81] which may influence long-term risk of development of type 2 diabetes and cardiovascular disease.[82,83] Insufficient vitamin B_{12} status has an important negative influence on children's development and cognitive functioning.[84,85] Nutritional vitamin B_{12} deficiency in children, and a late diagnosis of this condition, may lead to irreversible neurologic damage such as growth and motor retardation and even convulsions,[86] and recent studies indicate a relationship between maternal vitamin

B_{12} status and bone mass in the offspring.[87] Disturbed vitamin B_{12} status as well as (maternal) genetic factors may influence DNA methylation patterns in the newborn and thereby predispose individuals to specific disease later in life.[88]

NATURAL COURSE OF VITAMIN B_{12} DEFICIENCY

Importantly, the natural course of vitamin B_{12} deficiency is not clearly understood. In typical patients, in whom clear signs and symptoms associated with (poly)neuropathy and/or megaloblastic anemia are explained by the unequivocal finding of low serum vitamin B_{12} levels, like in patient A, it is not particularly difficult to make a diagnosis and institute treatment. However, more widespread screening for vitamin B_{12} deficiency may result in situations in which low serum vitamin B_{12} levels are found but without clear symptomatology.[10] Parenteral vitamin B_{12} administration is also used—according to the package insert—for the prevention of vitamin B_{12}–associated problems. In some individuals without symptoms, low levels of serum vitamin B_{12} may be associated with reductions in the binding protein haptocorrin.[89] However, this condition may be difficult to discriminate from true vitamin B_{12} deficiency in the presence of symptoms, as is evidenced by patient B.

JUST A VITAMIN, OR MORE?

Some of the cases reported in this article, as well as the study by Smelt et al[66] mentioned previously, suggest that supplementation of vitamin B_{12} itself, regardless of the actual vitamin B_{12} status, improves clinical symptoms. This hypothesis may put our thinking on the pathophysiology of vitamin B_{12} deficiency in a different perspective. Maybe we should not regard vitamin B_{12} as a vitamin that needs to be restored to normal levels to ensure proper functioning of metabolic pathways but rather as a general nerve-protecting and nerve-regenerating compound. There are some clues in the medical literature that support this concept. Cobalamin may regulate the balance between neurotoxic and neurotrophic agents.[90,91] Micronutrients including vitamin B_{12} might improve the neuropathy score in patients with type 2 diabetes,[92] but in this study, a mixture of nutrients was used, including multivitamins and vitamin B_{12} orally. Another study reported that parenteral vitamin B_{12} administration was more effective than nortriptyline in the treatment of painful diabetic neuropathy.[93] A 2005 meta-analysis[94] reported that a high-dose oral cyanocobalamin or methylcobalamin had beneficial effects on symptoms of diabetic neuropathy, such as pain and paresthesia. In 3 studies, methylcobalamin therapy also improved autonomic symptoms. Effects on vibration perception and electrophysiologic measures were not consistent. However, one review did not find any evidence that the use of oral vitamin B_{12} supplements is associated with improvement in the clinical symptoms of diabetic neuropathy.[95] Low central nervous system cobalamin levels may play a role in the development of multiple sclerosis.[96,97] Similar observations, and even reports on beneficial effects of vitamin B_{12} injection therapy, have been reported in patients with myalgic encephalomyelitis, with and without fibromyalgia.[98,99] Also, there are clues that low vitamin B_{12} status is related to the development of chemotherapy-induced peripheral neuropathy.[100] Prospective studies to assess whether vitamin B_{12} supplementation may prevent chemotherapy-induced peripheral neuropathy are planned or ongoing.[101]

A recent intriguing study comes from US investigators, who demonstrated low vitamin B_{12} levels in the brain tissue of aged individuals, especially those older than 60 years, and of people with autism and schizophrenia.[102] They speculate that on one hand vitamin B_{12} status in the brain compartment is distinctly regulated during aging from the rest of the body, while on the other hand may contribute to impaired brain function and in the etiology of neurologic disorders. These observations are supported by a recent study that reported improvement of symptoms in children with autism spectrum disorder treated with frequent hydroxocobalamin injections.[103] Finally, in a prospective study, Brito et al[104] found that one injection of high-dose (10 mg) cyanocobalamin, pyridoxine, and thiamine increased several metabolic markers of mitochondrial function oxidative stress, nerve function, and myelin integrity such as acylcarnitines, plasmalogens, phospholipids, and sphingomyelins.

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HOW SHOULD TREATMENT BE GIVEN?

Traditionally, vitamin B_{12} is administered by intramuscular (IM) injection because of the low degree of resorption after oral administration as a consequence of the underlying disease. For parenteral therapy in cases of megaloblastic anemia, it has been advised to administer 1000 μg hydroxocobalamin IM twice weekly for a period of 5 weeks, and the dose is reduced to 1000 μg IM every 2 months thereafter. In the United States, often cyanocobalamin is used.[10,15] In case of neurologic symptoms or abnormalities, it is suggested to administer hydroxocobalamin, 1000 μg once or twice weekly for a period of up to 2 years, and the package insert for hydroxocobalamin has included these particular instructions for several decades. However, it is ill defined which neurologic symptoms or abnormalities require such intensive treatment. Clinical practice has shown that in a substantial number of patients seen in a tertiary care setting, injection frequency cannot be reduced after the initial loading regimen. This topic has gained much interest recently, and studies evaluating this phenomenon are ongoing.[105]

Since the 1990s, interest in the possibilities of oral instead of parenteral administration has emerged. In 2014, a Dutch Viewpoint on Vitamin B_{12} Treatment was presented by the Dutch Organization of General Practitioners.[106] Motivation to publish this Viewpoint was the observed increase in serum vitamin B_{12} testing and findings of abnormal test results. In this Viewpoint, it was stated that oral vitamin B_{12} administration was preferred over parenteral supplementation. This advice has been based on a very limited number of clinical trials. A 2005 Cochrane review evaluated 2 randomized controlled trials comprising a total of 108 participants and suggested that oral vitamin B_{12} may be as effective as IM administration in obtaining short-term hematologic and neurologic responses in vitamin B_{12}–deficient patients.[107] The first study by Kuzminski et al[108] was performed in 33 vitamin B_{12}–deficient patients, the majority with atrophic gastritic or pernicious anemia, and reported that serum vitamin B_{12} levels were significantly higher and MMA levels significantly lower in the oral group (n=18) compared to the IM group (n=15). Similar numbers of patients reported improvement (n=2) or clearance of symptoms (n=2) in both groups. Typically in patients using vitamin B_{12} injections, serum vitamin B_{12} levels exceed the upper limit of normal (1476 pmol/L), and this finding suggests that there may be underdosing in the injection group. Patients were randomized to receive 2000 μg of oral cyanocobalamin administered with breakfast daily for 4 months or 1000 μg of cyanocobalamin administered IM with increasing intervals. Evaluation was at 4 months, when the oral group was still using supplementation but the IM group had received the most recent injection 1 month earlier. In addition, parenteral administration consisted of cyanocobalamin, not hydroxocobalamin, and it has been reported that hydroxocobalamin has a longer retention in plasma and a lower excretion in urine than an equivalent dose of cyanocobalamin.[109] A second study performed in Turkey[110] included 70 patients with megaloblastic anemia. In 8 of 24 participants in the oral group, vitamin B_{12} deficiency was caused by poor nutrition. Ten patients were excluded because they did not appear for follow-up, and it should be noted that these patients were mainly randomized to the oral supplementation group. Research into the etiology of vitamin B_{12} deficiency was rather poor. Only 19 patients had anti–parietal cell antibodies assessed, which was due to costs as stated by the authors. Finally, the prevalence of neurologic symptoms was low. Only 7 of 60 participants reported altered cognitive function. Taken together, the articles provide very weak support for oral rather than parenteral vitamin B_{12} administration, and the large number of participants in the study by Bolaman et al[110] with an etiology of poor nutrition (and not malabsorption) strongly biases these results in favor of simple oral vitamin B_{12} administration, which is the default in any nutritional insufficiency of vitamin B_{12} including vegetarianism. However, dosage is important in this respect. A study by Hill et al[21] found that supplementation with 500 μg of cyanocobalamin did not normalize moderately elevated MMA levels in healthy elderly people (aged 65 years or older). A recent Cochrane review confirmed

that there is only very low-quality evidence that oral vitamin B_{12} appears as safe as IM vitamin B_{12}.[111] The authors concluded that further trials should conduct better randomization and blinding procedures, recruit more participants, provide adequate reporting, and measure important outcomes such as the clinical signs and symptoms of vitamin B_{12} deficiency, health-related quality of life, socioeconomic effects, and adverse events adequately.[111] Serious adverse effects, even with high doses of hydroxocobalamin, have never been reported.[112,113] However, some patients do report acneiform eruptions or rosacea.[114]

POSSIBLE PLACEBO EFFECTS UNLIKELY

In discussions on the effect of hydroxocobalamin treatment, often the suggestion is made that part of the effect is that of a placebo. Indeed, there is evidence that placebo treatments can have large and sustained effects on clinical outcomes in multiple disorders, as reviewed by Ashar et al,[115] who suggested that this effect may be particularly prominent in disorders in which emotion and motivation play a central role. In vitamin B_{12} deficiency, there is no evidence that recovery of megaloblastic anemia or normalization of elevated MMA levels as a consequence of hydroxocobalamin injections can be regarded as a placebo effect. Yet, when patients' neurologic or cognitive symptoms improve by injection therapy, it is often ascribed to a placebo effect. Several studies have assessed the effects of oral vitamin B_{12} supplementation in a variety of disorders, usually in people with normal serum vitamin B_{12} levels,[116,117] with a surprising benefit in, for example, aphthous stomatitis.[118] We have not found many well-designed studies of hydroxocobalamin injections in patients with neurologic symptoms caused by vitamin B_{12} deficiency. One study reported favorable effects of hydroxocobalamin injections in patients with mechanical or irritative lumbago,[119] whereas another well-designed study reported improvement of symptoms in children with autism spectrum disorder.[103] Clearly, there is a need for well-conducted double-blind, randomized studies in patients with vitamin B_{12} deficiency who report the benefit of continued frequent (twice weekly) hydroxocobalamin injections vs oral supplementation.

CONCLUSION

The spectrum of symptoms and signs suggestive of vitamin B_{12} deficiency is reasonably well defined. Nevertheless, many of the symptoms are nonspecific and may occur as a consequence of other diseases. Currently, no research has documented the positive and negative predictive values of specific symptoms or symptom scores for the presence of vitamin B_{12} deficiency.

Patients with low serum vitamin B_{12} levels may have no symptoms (yet). Nevertheless, they are at high risk for development of symptoms. There is a tendency among physicians to consider a serum vitamin B_{12} level higher than 140 pmol/L as normal, but many symptomatic patients may present with such levels, for instance because of taking oral vitamin supplementation. This does not mean that their tissue vitamin B_{12} levels are normal as well. Methylmalonic acid and homocysteine are not very sensitive biomarkers, but there is currently no good alternative, although systematic evaluation of more advanced metabolic factors may lead to the application of better biomarkers. When serum total vitamin B_{12} levels are low or questionable, the combination of total vitamin B_{12}, active vitamin B_{12}, MMA, and homocysteine may be the best strategy, but the validity of this combined biomarker approach[43] needs to be validated in larger prospective studies and especially validated against objective markers of treatment response. In case of doubt, when results of biomarker measurements are equivocal, a trial with parenteral hydroxocobalamin injections may be considered, as was done in patients B and D. Because symptom improvement in long-standing (subclinical) vitamin B_{12} deficiency may take some time, we usually advise a treatment regimen of twice weekly hydroxocobalamin injections for 3 months, after which a thorough reevaluation is performed with systematic evaluation of symptom score as demonstrated in patient B (Table 2). There is no proof in large prospective, double-blind studies that oral supplementation is as effective in reducing symptoms associated

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with vitamin B_{12} deficiency as parenteral treatment.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://mcpiqojournal.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: **CoA** = coenzyme A; **holoTC** = holotranscobalamin; **IF** = intrinsic factor; **IM** = intramuscularly; **MMA** = methylmalonic acid

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